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ORIGINAL ARTICLE

Transmission of *Salmonella enterica* serotype Typhimurium in poultry with and without antimicrobial selective pressure

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Abstract

Aims: To determine the effect of antimicrobial selective pressure on the transmission of antimicrobial resistant and sensitive strains of *Salmonella* in poultry.
Methods and Results: Eight pens housed 12 broiler chicks each. Two chicks in four of the pens were inoculated with a *Salm.* Typhimurium strain resistant to 12 antimicrobials (including tetracycline), and two chicks in each of the four other pens were inoculated with a strain sensitive to all antimicrobials tested. Two pens inoculated with each strain were treated with chlortetracycline and two were not. Chicks were killed on day 7 and caeca were cultured for *Salmonella*. Experiments were performed independently twice. Chicks exposed to pen mates inoculated with the resistant strain and treated with tetracycline were 90% positive for *Salmonella*; whereas 60% of chicks given no antimicrobials were positive. Chicks exposed to the sensitive strain were 95% positive with tetracycline treatment and 90% positive without treatment.
Conclusions: A multidrug-resistant *Salm.* Typhimurium strain had significantly increased transmission when chicks were treated with tetracycline. Transmission of a sensitive strain was not inhibited by antimicrobial selective pressure at recommended therapeutic dose.
Significance and Impact of the Study: This study demonstrates that antimicrobial usage may influence the transmission of antimicrobial-resistant pathogens in poultry.

Introduction

Food-borne disease caused by *Salmonella* remains a major health concern in the USA as well as other countries. The Centers for Disease Control and Prevention (CDC) estimated that infections from *Salmonella* affected 1·4 million people in the USA in 1999, 22% of them were hospitalized and 0·8% died (Guerri *et al.* 2004). Although the majority of *Salmonella* infections are self-limiting diarrhoeal illnesses, severe and even life-threatening infections that require antimicrobial treatment do occur. Further complicating food-borne infections, *Salmonella enterica* serotype Typhimurium (*Salm.* Typhimurium) appear to be more capable of developing resistance to antimicrobials and many *Salmonella* are resistant to multiple antimicrobials.

As the chicken intestinal tract can contain *Salm.* Typhimurium at a subclinical level, controlling *Salmonella* in food-producing animals is of great concern due to the high rate of food contamination (Salyers 2002). *Salmonella* Typhimurium with resistance to one or more antimicrobials are believed to be more virulent and more likely to cause septic shock and in severe cases the individual may need antimicrobial treatment (Varma *et al.*

2005). In addition, multiple antimicrobial-resistant *Salmonella* may also respond poorly to antimicrobial treatment (Carlson *et al.* 2001). In 1980, 13% of *Salm.* Typhimurium responsible for human infections were resistant to one or more antimicrobials and this percentage increased to 51% in 2001 (Varma *et al.* 2005). In 2002, *Salm.* Typhimurium was the most common *Salmonella* serotype found among animal species as reported by the National Antimicrobial Resistance Monitoring System (NARMS) (Fedorka-Cray *et al.* 2003). Of the 393 *Salm.* Typhimurium tested, 27% were found to be resistant to five or more antimicrobials while 40% were resistant to at least one antimicrobial (NARMS) (Fedorka-Cray *et al.* 2003). Based on data collected by FoodNet (CDC, Atlanta, GA, USA) from 1997 to 1998, it was estimated that *Salmonella* infections accounted for 26% of hospitalizations for food-borne illness and 0·6% of these people died from the infection (Mead *et al.* 1999).

Salmonella with resistance to fluoroquinolones and extended-spectrum cephalosporins have been uncommon in the USA. Therefore, these antimicrobials have become the suggested antimicrobial agents for the treatment of severe *Salmonella* infections (Shea and The Committee on Environmental Health and Committee on Infectious Diseases 2004). Resistance to extended-spectrum cephalosporins has been found in 18·8% of the *Salmonella* animal isolates from North America in 2003 (http://www.ars.usda.gov/Main/docs.htm?docid=6750). This resistance has been linked to a plasmid-mediated CMY-2 AmpC-like beta-lactamase (bla_{CMY-2}) that hydrolyses cephalosporins (Winokur *et al.* 2000; Gray *et al.* 2004). The bla_{CMY-2} gene is located on a transferable plasmid with multiple drug resistance and has been found in 21 different serotypes of *Salmonella* (Gray *et al.* 2004). Tetracycline resistance was also found in 100% of the bla_{CMY-2} gene-positive *Salmonella* (Gray *et al.* 2004). Many genes for antimicrobial resistance can be genetically linked to other genes that confer resistance to different antimicrobials, similar to this association between tetracycline and extended-spectrum cephalosporins (Aminov *et al.* 2001).

The emergence of resistance in *Salmonella* has been associated with the use of antimicrobial agents in livestock, including chickens (Fedorka-Cray *et al.* 1994; Gray *et al.* 1996; Bell and Weaver 2002; Salyers and Whitt 2005). It is not known whether the spread of multiple drug-resistant *Salmonella* is caused by an increase in virulence due to the selective pressure from antimicrobial treatment. Furthermore, there are few experiments that clearly study virulence traits, such as transmission, of a resistant strain of *Salm.* Typhimurium in an animal model. To explore the relationship between *Salm.* Typhimurium resistance and a competitive advantage for colonization under antimicrobial selective pressure, we examined the transmission of multidrug resistant and sensitive *Salm.* Typhimurium with and without antimicrobial selective pressure in a poultry model.

Materials and methods

Bacterial strains and growth conditions

The two strains of *Salm.* Typhimurium used in this study were obtained from farms in the USA for antimicrobial susceptibility testing as part of the National Antimicrobial Resistance Monitoring program (http://www.ars.usda.gov/main/site_main.htm?modecode=66120508). The resistant strain (8381r) was resistant to 12 of the 17 antimicrobials indicated in Table 1. Strain 8382s was sensitive to all antimicrobials listed in Table 1. Nalidixic acid (Sigma, St Louis, MO, USA) was chosen as a selective marker for the recovery of the experimental strains from the background microflora. Nalidixic acid resistance was induced and selected for by passing the strains on Luria-Bertani [LB Agar, Difco Cat# 244520; Becton Dickinson (BD), Sparks, MD USA] agar plates containing increasing concentrations of nalidixic acid. Briefly, isolates of 8381r and 8382s were grown on LB plates containing 16 μg ml^{-1} of nalidixic acid for 24 h at 37°C. Isolates that grew were subsequently streaked onto LB plates containing 32 and

Table 1 Antimicrobial susceptibility of *Salmonella* Typhimurium 8381r and 8382s

Antimicrobial	8381r	8382s
Amikacin	s*	s
Amoxicillin/clavulanic acid	R†	s
Ampicillin	R	s
Apramycin	s	s
Cefoxitin	R	s
Cetfiofur	R	s
Ceftriaxone	s	s
Cephalothin	R	s
Chloramphenicol	R	s
Ciprofloxacin	s	s
Gentamicin	R	s
Imipenem	s	s
Kanamycin	R	s
Nalidixic acid	R‡	R‡
Streptomycin	R	s
Sulphamethoxazole	R	s
Tetracycline	R	s
Trimethoprim/ Sulphamethoxazole	R	s

*s, Susceptible to antimicrobial as defined by CLS breakpoint standards.
†Resistance to antimicrobial as defined by CLS breakpoint standards.
‡Resistance to nalidixic acid was induced in *Salm.* Typhimurium 8381r and 8382s.

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64 μg ml^{-1} nalidixic acid. Nalidixic acid-resistant isolates were then passed three times on 32 μg ml^{-1} nalidixic acid, followed by six successive passages on plates without antimicrobials. To verify resistance, the isolates were streaked again on plates containing 32 μg ml^{-1} nalidixic acid and then stored at −80°C. To ensure that this process had not caused any other changes in antimicrobial susceptibility, these strains were assayed for resistance (Table 1) to antimicrobials as described below.

For inoculation of seeder chicks, both the strains were grown overnight in 2·5 ml of LB broth (LB liquid, Difco Cat# 244620; BD) for approx. 17 h at 37°C in a shaking water bath at 180 rev min^{-1}. One millilitre of each overnight culture was transferred into a 150-ml fresh LB broth and placed into the shaking water bath for 5 h. The optical density at 600 nm (OD_{600}) was checked with a spectrophotometer and the culture was diluted with PBS (phosphate-buffered saline, Cat# 161–0780; Bio-Rad Lab., Hercules, CA, USA) to obtain an OD_{600} of 0·8 corresponding to 10^9 colony forming units (CFU) ml^{-1}. Isolates were enumerated by spiral plating onto Briliant Green Sulfar agar (BGS agar, Difco Cat# 271710; BD), with 32 μg ml^{-1} nalidixic acid with spiral auto-plater (Spiral Biotech, Bethesda, MD, USA). Colonies were counted using a Q count (Spiral Biotech).

Determination of antimicrobial susceptibility

Antimicrobial susceptibility testing was performed using a semi-automated broth microdilution system (Sensititre; TREK Diagnostics, Inc., Westlake, OH, USA) as per the manufacturer's directions. Custom 96-well plates were used from the NARMS program (http://www.ars.usda.gov/main/site_main.htm?modecode=66120508) and included antimicrobials used in both human and veterinary medicine. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) procedures were followed throughout the testing procedure and CLSI breakpoints were used for interpreting results (NCCLS 2002). Isolates were examined for susceptibility to the antimicrobials listed in Table 1. *Esherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Susceptibility of the inoculated *Salm.* Typhimurium 8381r and 8381s was evaluated pre- and postexposure to nalidixic acid and is listed in Table 1.

Animals

A total of 192 day-of-hatch broiler chicks from a commercial hatchery were randomly separated into two biosecure rooms with four pens in each room (groups of 12 birds each) as shown in Fig. 1. All birds were from the same parent flock, a single hatchery incubator, and a single delivery crate to minimize variability. Pens were separated by three-foot high solid panels that prevented any wood-chip litter or birds from crossing into other pens. The animal technicians' protective equipment, including gloves, boots and gowns, were changed between handling birds in different pens to minimize the risk of cross-contamination by staff movements. The chicks were assayed for contamination with nalidixic acid-resistant *Salmonella* before the start of the experiments by plating swabs from the shipping container onto BGS media with 32 μg ml^{-1} nalidixic acid. No *Salmonella* with nalidixic acid resistance were recovered. Each pen had separate feed (Purina, St Louis, MO, USA) and water supplied *ad lib*. Each bird was marked on the back with a colour to distinguish them from each other and for future record keeping.

Experimental design

Figure 1 shows a diagram of pens, broiler chicks, *Salm.* Typhimurium strains and treatments used in these experiments. Chicks were placed in two bio-secure rooms with each room exposed to either 8381r or 8382s. The rooms were further divided into four pens containing 12 birds each. Two pens in each room received a constant dose of chlortetracycline (tet; Fort Dodge Animal Health, Fort Dodge, IA, USA) in drinking water while the other two pens in each room received no antimicrobials, for a total of four treatments (8381r and 8382s with and without tet). The experiments were repeated twice, thus four replicates of each treatment were performed. Fresh water with or without tet was supplied daily. For chicks treated with tet, 120 μg of chlortetracycline per ml of water was added every 24 h according to the manufacturer's directions for therapeutic dose of this antimicrobial in chickens. Prior to initiation of treatment, two seeder chicks from each pen were inoculated with 10^9 CFU ml^{-1} of 8381r or 8382s *per os*. Uninoculated broiler chicks were killed by cervical dislocation and necropsied on day 7 postinoculation for examination of the caecum.

Recovery, identification and quantification of bacteria from uninoculated broiler chicks

A total of 1 g from the two caeca sampled was placed in bags with 50 ml of PBS. Qualitative bacteriology was performed by stomaching with a Mini Mix (Interscience, Suarlee, Belgium) for 2 min and placing 100 μl into GN (Gram negative, Hajna) broth (Difco Cat# 248610; BD) containing 32 μg ml^{-1} nalidixic acid. Samples were incubated at 37°C for 18 h. Presumptive positive isolates, indicated by turbidity, were plated onto BGS plates. For

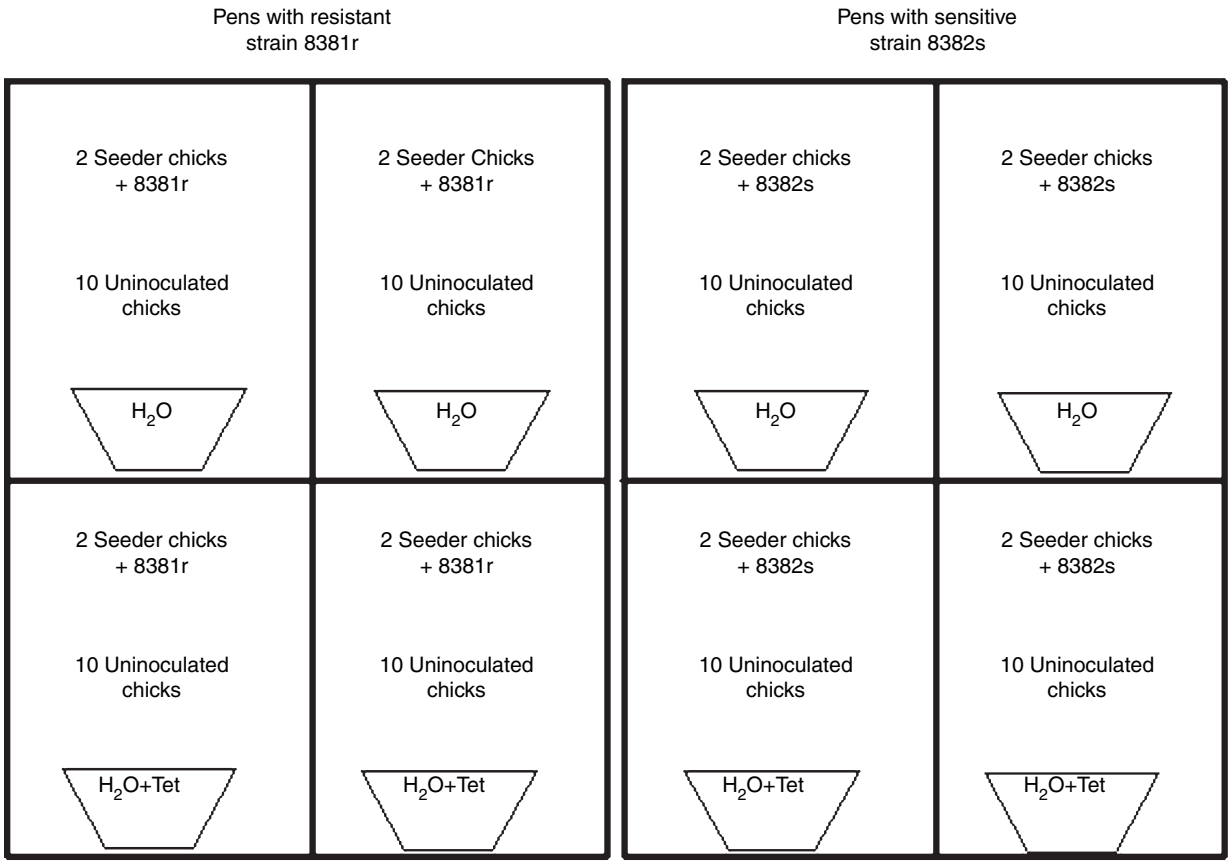

Figure 1 Diagram of pens, broiler chicks, *Salmonella* Typhimurium strains and treatments used in these experiments. Two bio-secure rooms were each divided by three-foot high impervious plastic partitions into four pens. Seeder chicks in the pens of the left room were inoculated with the multidrug-resistant strain 8381r. Two pens were untreated (H_2O) and two pens were treated with tetracycline in drinking water (H_2O + tet; 120 μg ml^{-1}). Seeder chicks in the pens on the right were inoculated with the sensitive strain, 8382s. Two pens were untreated and two pens were treated with tetracycline in drinking water (120 μg ml^{-1}). The entire experiment was repeated on a different week with another set of broiler chicks.

further identification, isolates were inoculated onto triple sugar iron agar (TSI Cat# 0265–17–1; BD) slants and lysine iron agar (LIA Cat# 0849–17–6; BD) slants and xylose lactose Tergitol (XLT-4), agar (Cat# C803; Hardy-Diagnositics, Santa Maria, CA Hardy Diagnostics, Santa Maria, CA, USA) and scored by appearance. To confirm that the *Salmonella* isolates recovered from uninoculated chicks were similar to those used to inoculate the seeder chicks, 10 isolates were selected randomly from each of the four treatments for further analysis. Isolates were sent to the National Veterinary Services Laboratories (NVSL; Ames, IA, USA) for serotyping. Antimicrobial susceptibility was determined as described above.

Quantitative bacteriology of caeca was performed by spiral autoplating (Spiral Biotech), the homogenized caecum suspended in PBS onto BGS plates with 32 μg ml^{-1} nalidixic acid and incubated at 37°C for 18 h. The Q count imaging system was used for quantitative analysis. Further analysis of presumptive positives was performed as described for the qualitative analysis. The weights of caeca collected were determined prior to homogenation in order to calculate the CFU g^{-1}.

Statistical analysis of the data

Prior to analysis, CFU g^{-1} of bacterial counts was logarithmically transformed. Differences in *Salmonella* transmission and numbers in the organs were determined using the analysis of variance (SAS/English statistical package version 9.1; Cary, NC, USA) with significance expressed at $P \leq 0{\cdot}05$.

PCR and pulsed field gel electrophoresis (PFGE) analysis

Polymerase chain reaction analysis was performed to determine the presence of the $bla_{\text{CMY-2}}$ gene in *Salm.* Typhimurium strains 8381r and 8382s as described by Gray *et al.* (2004). To confirm recovered isolates were identical

to inoculated isolates, PFGE analysis was performed as previously described (Sambrook and Russell 2001) on nine of the isolates recovered from uninoculated chicks that became colonized during the course of the experiment.

Results

Antimicrobial susceptibility of challenge strains

For this study, *Salm.* Typhimurium strains were selected from isolates collected as part of the NARMS program. The multidrug-resistant (MDR) strain, 8381r, was found to be resistant to a third-generation cephalosporin, ceftiofur, and tetracycline as well as 10 other antimicrobials (Table 1). Strain, 8382s, was sensitive to 17 antimicrobials assayed. To separate them from other natural flora in the broiler chicks, nalidixic acid resistance was selected for in each strain, and each strain was assayed again for susceptibility to antimicrobials. Both strains displayed the original susceptibility patterns, but were now resistant to nalidixic acid (Table 1). PCR analysis was used to determine whether the $bla_{\text{CMY-2}}$ gene was present in the ceftiofur-resistant strain MDR, 8381r. The gene was detected in the resistant strain 8381r, but not in the sensitive strain 8382s (data not shown).

Effect of chlortetracycline on the transmission of the resistant strain from inoculated seeder chicks to uninoculated pen mates

Prior to the start of the study, the broiler chicks obtained for these experiments were assayed for *Salmonella* resistant to nalidixic acid. No *Salmonella* with nalidixic acid resistance were recovered from the chickens, indicating that there was no detected exposure to nalidixic acid-resistant *Salmonella* at the hatchery prior to exposure to the challenge strains. At day 7 after inoculation of the seeder chicks, the caeca of all uninoculated chicks were examined to determine whether the resistant strain of *Salm.* Typhimurium, 8381r, had colonized these birds. The number of chicks positive for 8381r is listed in Table 2. After commingling with the 8381r-infected seeder birds, 90% (36/40) of the uninoculated pen mates treated with chlortetracycline became colonized with 8381r. Without tetracycline treatment, 60% (24/40) of uninoculated pen mates became colonized with 8381r as shown in Table 2. The transmission of the MDR-resistant strain 8381r was significantly higher ($P = 0{\cdot}03$) in chicks that received a therapeutic dose of chlortetracycline (120 μg ml^{-1}) compared with those that did not receive chlortetracycline. To determine whether chlortetracycline treatment had an effect on the number of the resistant bacteria within the chicks, the average CFU 8381r in the caeca of the birds was found by plate count. The mean number of *Salm.* Typhimurium 8381r was significantly greater ($P = 0{\cdot}0001$, by ANOVA) in birds treated with chlortetracycline than those not treated [4·7 CFU ml^{-1} ($\log_{10}$) and 2·8 CFU ml^{-1} ($\log_{10}$) respectively].

Effect of chlortetracycline on the transmission of the sensitive strain from inoculated seeder chicks to uninoculated pen mates

The sensitive strain 8382s was recovered from 95% (38/40) of chickens treated with chlortetracycline and from 90% (36/40) of the chickens not treated with chlortetracycline (Table 2). There was no significant difference between the two treatments. The mean number of *Salm.* Typhimurium 8382s was 6·11 CFU ml^{-1} ($\log_{10}$) in birds treated with chlortetracycline and 5·7 CFU ml^{-1} ($\log_{10}$) in those not treated, and no significant difference ($P = 0{\cdot}30$) was observed between the two treatments.

Characterization of *Salmonella* isolates recovered from uninoculated broiler chicks caeca

To determine whether the nalidixic acid-resistant *Salmonella* isolates recovered postchallenge from the uninoculated chicks matched those used to inoculate the seeder chicks, 10 isolates were selected randomly from each of the four treatment groups ($n = 40$). None of the isolates from chicks exposed to the resistant strain, 8381r, demonstrated any change in resistance pattern regardless of treatment. However, three of 20 isolates recovered from chicks challenged with the sensitive strain, 8382s, and treated with chlortetracycline became resistant (Table 3). Three of these isolates were resistant to tetracycline, gentamicin, streptomycin and sulphamethoxazole (these isolates are labelled

Table 2 Recovery of *Salmonella* Typhimurium 8381r or 8382s from the caeca of uninoculated broiler chicks with and without chlortetracycline treatment

Treatment	8381r Positive chicks/ total chicks (%)	8382s Positive chicks/ total chicks (%)	8381r CFU g^{-1}($\log_{10}$)	8382s CFU g^{-1}($\log_{10}$)
Chlortetracycline	36/40 (90%)	38/40 (95%)	4·7	6·1
No chlortetracycline	24/40 (60%)	36/40 (90%)	2·8	5·7
P-value (significant at $P < 0{\cdot}05$)	0·03	0·31	0·0001	0·30

Table 3 Antimicrobial resistance in isolates from chicks challenged with sensitive strain of *Salmonella* Typhimurium, 8382s, with and without chlortetracycline treatment

Isolate	Treatment	Antimicrobial*
8382s-a	No antimicrobials	Sulphamethoxazole
		Trimethoprim/sulphamethoxazole
8382s-b	No antimicrobials	Gentamicin
		Sulphamethoxazole
		Tetracycline
8382s-c	Chlortetracycline	Gentamicin
		Streptomycin
		Sulphamethoxazole
		Tetracycline
8382s-d	Chlortetracycline	Gentamicin
		Streptomycin
		Sulphamethoxazole
		Tetracycline
8382s-e	Chlortetracycline	Gentamicin
		Streptomycin
		Sulphamethoxazole
		Tetracycline

*Resistance to antimicrobial indicated as defined by CLS breakpoint standards.

8382s-c, d and e in Table 3 and Fig. 2). In addition, two of 20 isolates recovered from caeca of birds not treated with chlortetracycline became resistant. However, only one of these two isolates became resistant to tetracycline (Table 3 isolates 8382s-a sulfamethoxazole and trimethoprim/sulphamethoxazole, and 8382s-b tetracycline, gentamicin and sulphamethoxazole). All of the isolates from chicks challenged with 8382s that became resistant to antimicrobials were serogrouped and serotyped as Typhimurium. PFGE profiles of resistant 8382s isolates a, b, c, d, and e generated indistinguishable profiles matching the inoculated 8382s strain (Fig. 2). PFGE profiles were also indistinguishable for the isolates recovered from chicks colonized by the resistant strain, 8381r (Fig. 2.)

Discussion

The use of antimicrobials in livestock production has been linked to the development of antimicrobial resistance in bacteria and resistant *Salmonella* can create therapeutic challenges for humans and animals infected by these bacteria. MDR *Salmonella* are believed to be

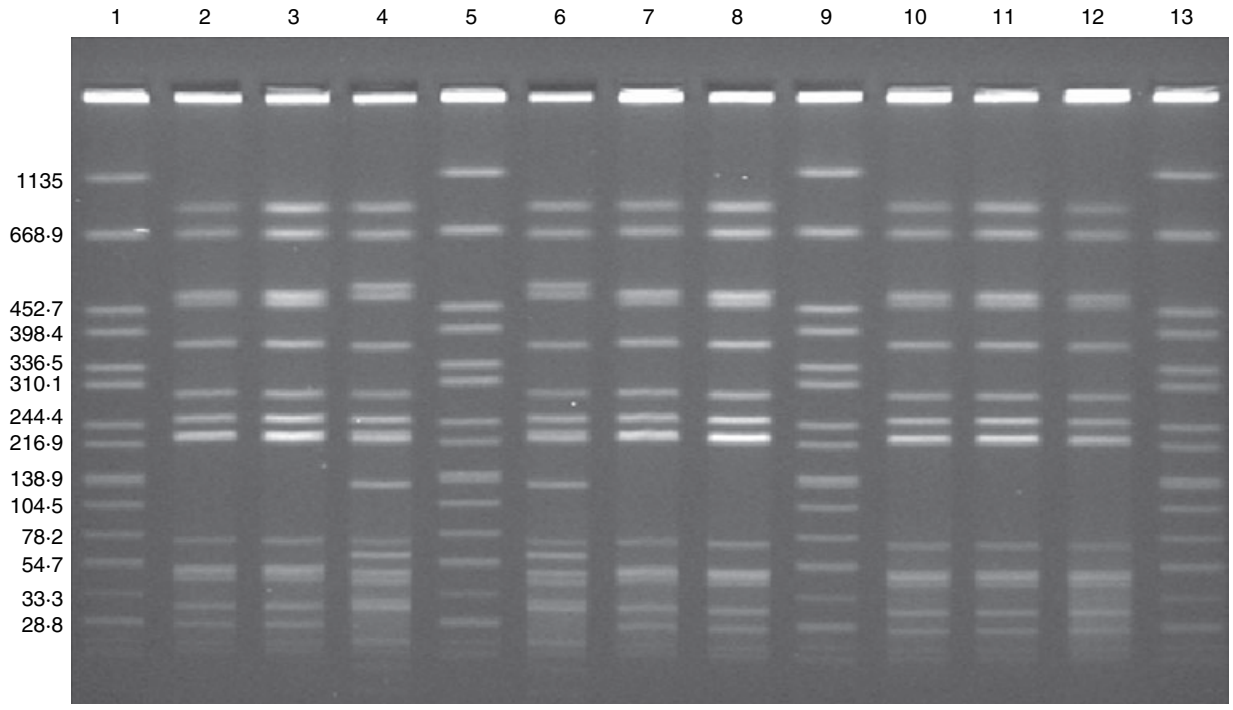

Figure 2 PFGE patterns of *Xba*I digested DNA from strains 8381r and 8382s and isolates recovered from chicks colonized during the transmission study. Lane 1, control-H9812 (*Salmonella* serotype Branderup); lane2, 8382s-c (isolated from chlortetracycline-treated chicks); lane 3, 8382s-f (isolated from chlortetracycline-treated chicks); lane 4, 8381r-a (isolated from untreated chicks); lane 5, H9812; lane 6, 8381r inoculum; lane 7, 8382s-d (isolated from chlortetracycline-treated chicks); lane 8, 8382s inoculum; lane 9, H9812; lane 10, 8382s-e (isolated from chlortetracycline-treated chicks); lane 11, 8382s-b (isolated from untreated chicks); lane 12, 8382s-a (isolated from untreated chicks); lane 13, H 9812.

more virulent under antimicrobial selective pressure, which may allow them to spread more rapidly from animal to animal (Barza and Travers 2002). This study was designed to determine whether antimicrobial selective pressure would affect the transmission of MDR *Salmonella*. The results of this study show that in broiler chicks, MDR *Salm.* Typhimurium significantly increased transmissibility under antimicrobial selective pressure. Within 7 days, 90% (36/40) of the birds treated with a therapeutic dose of chlortetracycline became colonized with the MDR strain of *Salm.* Typhimurium compared with only 60% (24/40) of the untreated control birds. Furthermore, the number of resistant *Salm.* Typhimurium found in the caecum was significantly (by approx. 4 logs) higher in the birds treated with chlortetracycline than in those not treated with antimicrobials. Birds not treated with chlortetracycline had significantly fewer challenge bacteria residing within their organs; thus indicating the overall *Salmonella* load on the flock was lower which may have led to reduced transmission. It is believed that antimicrobials disrupt the microflora of animal intestines by reducing sensitive strains of bacteria allowing the resistant strain to overpopulate that region (Yan and Gilbert 2004). In this experiment, higher numbers of bacteria found within the caeca under antimicrobial selective pressure support this concept, and higher numbers of *Salm.* Typhimurium shed into the environment could explain the increased colonization between the birds.

Interestingly, in broiler chicks challenged with a sensitive *Salm.* Typhimurium strain, therapeutic levels of chlortetracycline did not prevent the transmission of the isolate as 95% of the chicks treated with chlortetracycline were colonized. Further, mean numbers of the sensitive *Salm.* Typhimurium within the caeca were approximately the same regardless of the treatment. This strongly suggests that antimicrobial treatment does not affect colonization of sensitive strains of Typhimurium and that virulence may not be linked to presence of resistance genes. Further genetic analysis of these strains of *Salm.* Typhimurium may reveal additional virulence or fitness factors in the sensitive strain that are not present in the resistant strain.

Antimicrobials that are used at subtherapeutic levels in the diet of livestock are considered too low to affect the growth of the bacteria, but high enough to allow the bacteria to accumulate mutations or acquire new DNA for resistances under the selective pressure particularly to the antimicrobial being used (Bjorkman and Andersson 2000). Antimicrobials used in livestock at levels designed to kill the bacteria, or therapeutic levels, are believed to be better for preventing the development of antimicrobial resistance (Hardy 2002). Interestingly, the therapeutic levels of chlortetracycline used in this experiment did not kill or deter colonization of the sensitive *Salm.* Typhimurium strain. Additionally, a low percentage of isolates recovered (15%, 3/20) became resistant to tetracycline as well as other antimicrobials, thus altering a sensitive isolate to multidrug resistance to additional antimicrobials in only 7 days. Levy *et al.* (1976) found that sensitive bacteria treated with therapeutic levels of tetracycline acquired a plasmid that conferred resistance to multiple antimicrobials within 1 week. Although we did not test for resistance in other populations of bacteria, it is possible that a mobile genetic element(s) could have been acquired by the five resistant strains isolated during this study.

Since the discovery of tetracycline in the 1940s, acquisition of tetracycline resistance genes has increased in pathogenic bacteria (Chopra and Roberts 2001). Tetracycline resistance in pathogenic bacteria that reside in poultry is a common occurrence and can be transmitted easily on a plasmid containing other resistant genes (Bjorkman *et al.* 2000). Obtaining chicks that are bacteria free or containing bacteria sensitive to all antimicrobials is almost impossible, thus the development of resistance observed in the sensitive strain of *Salm.* Typhimurium 8382s is likely to occur naturally in environmental bacteria. Previous studies have shown that development of resistance in bacteria not treated with antimicrobials is also a common incident that occurs at a much lower rate when compared with those under selective pressure (Levy *et al.* 1976). Because PFGE analysis did not detect any changes in the gross genotype of the isolates, future studies could attempt to detect and characterize any plasmids in the isolates that acquired resistance during this study.

Studies investigating the competition rate between sensitive and resistant *Salm.* Typhimurium in an animal model showed that the sensitive isolates were more virulent (Bjorkman *et al.* 2000). In the present experiment, the sensitive isolate colonized nearly 100% of the flock regardless of antimicrobial pressure. The sensitive isolate spread through the flock more efficiently than the resistant isolate when treated with an antimicrobial. One explanation for this phenomenon might be that *in vivo* antimicrobials did not target the sensitive strain of *Salm.* Typhimurium but killed or inhibited the growth of other more sensitive bacteria, most likely giving more room for this pathogen to grow. The sensitive strain had higher numbers of bacteria growing within the caeca of the birds when compared with the resistant strain with or without chlortetracycline treatment (Table 2). The higher numbers of bacteria within the caeca may have increased the amount of sensitive *Salm.* Typhimurium in the environment of broiler chicks. This could explain the higher colonization rate of the sensitive strain in broiler chicks when compared with the resistant isolate.

Concerns regarding the use of antimicrobials in livestock should not focus solely on the detrimental effects of

resistant strains of bacteria. In this study, the use of therapeutic levels of antimicrobials increased the colonization rate of resistant bacteria and had no effect on the colonization of the sensitive bacteria, but may have selected for the acquisition of resistance in the sensitive strain of *Salm.* Typhimurium. In conclusion, these experiments demonstrated that factors other than antimicrobial resistance and antimicrobial selective pressure are involved in the ability of a pathogen to colonize a flock of chickens and warrant further study.

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